

식물체에서 BCTV replicon 벡터 시스템을 이용한
재조합 GA733-2 단백질의 발현향상

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Enhanced expression of recombinant GA733-2 in plants
by BCTV replicon-based vector system

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Objective

The GA733-2 antigen is a 40-kDa human cell surface glycoprotein and has been found to be associated with human colorectal carcinomas. GA733-2 can be immunotherapeutic target of the most frequent human cancers. This antigen was chosen as a target protein to test the suitability of Beet curly top virus (BCTV) vector system.

Materials and Methods

○ Materials

A. tumefaciens GV3101, *N. bentamiana*

○ Methods

Plant tissue culture, plant transformation, DNA preparation,
Southern blot analysis, Western blot analysis

Results

Southern hybridization analysis showed that unit-length DNAs of replicated BCTV could be detected 3 and 6 days after the cultivation of *Agrobacterium*-inoculated leaf-disks of plants. Recombinant GA733-2 was expressed with a molecular size of

approximately 40 kDa in *Agrobacterium*-inoculated leaf-disks using a BCTV replicon-based expression vector system. Use of the ER retention signal in the BCTV vector system increased the expression of recombinant GA733-2, compared to use of the control vector. Expression of recombinant GA733-2 was further enhanced in the presence of post-transcriptional gene silencing suppressor p19. This work was supported by a grant from the Rural Development Administration through Bio-green 21 Project.

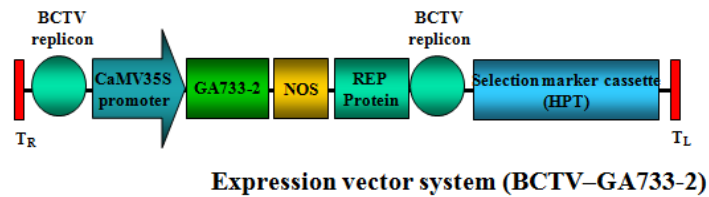


Fig. 1. Schematic diagram of the vector constructs.

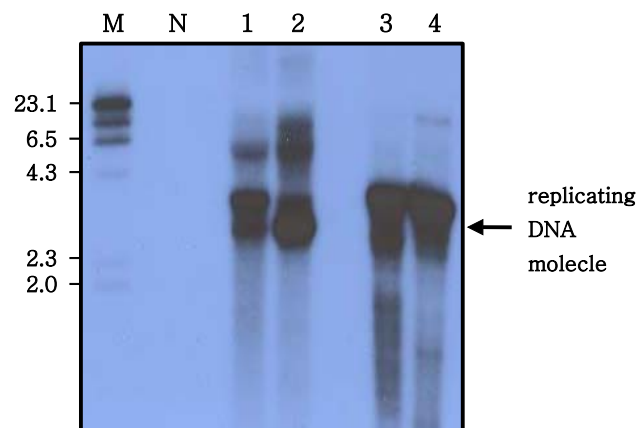


Fig. 2. Southern blot analysis of DNA extracts isolated from *Agrobacterium*-inoculated leaf-disks. M : λ Hind III marker. N : DNA samples isolated in normal *N. benthamiana* leaves. Lanes 1-2 : DNA samples isolated 3 and 6 days post-inoculation with recombinant *Agrobacterium*, Lanes 3-4 : DNA samples identical to lanes 1-2, but these DNAs are treated with restriction enzyme (Sal I)